COMPUTER SIMULATION OF DIFFERENTIAL KINETICS OF MAPK ACTIVATION UPON EGF RECEPTOR OVEREXPRESSION

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Abstract - **Everyday cells encounter various stimuli ranging from growth signals to bacterial infections to UV insult to death signals. They must somehow receive these signals, interpret them, integrate different stimuli, and generate the required output. They can do so by an intricate mechanism named intracellular signal transduction. There are various signal transduction pathways within a cell, each of which are designed for a particular stimulus, and all of which can crosstalk among themselves for the careful integration of all stimuli. In this report, only one of these pathways, the mitogen-activated protein kinase (MAPK) pathway has been simulated. This pathway is activated upon binding of growth factors to their respective cell surface-bound receptors. Activated receptors relay the incoming signal to the cell interior via a cascade of proteins, which are thought to be involved in both the amplification of the signal, and the specificity of the pathway. A generic MAPK pathway activated by the EGF (epidermal growth factor) and the effect of receptor overexpression has been studied, and consistent with experimental evidence, it is shown that the number of EGF receptors on the cell surface is a key factor in the response generated by the pathway.**

Keywords - **MAPK signaling, EGF, computer simulation**

I. INTRODUCTION

Cells can respond to various stimuli, ranging from growth and differentiation factors to stress signals such as ultraviolet (UV) or osmotic shock, through activation of the Receptor Tyrosine Kinases (RTKs) (Fig. 1a). Dimerization and autophosphorylation of the receptor is relayed to "adaptor" proteins that bind to the active receptor, whereby the signal is relayed to the MAPK cascade [1,2]. Activation of the MAPK cascade is achieved through localization of the cytoplasmic SOS protein to the plasma membrane by the RTK-associated adaptor proteins such as Grb2 (Fig. 1b) [3]. SOS can recruit Ras to the receptor and convert it into its active form (called Ras-GTP). The MAPK cascade is a series of enzymes that catalyze the phosphorylation of each other in turn: active Ras fires the pathway by activating the first "kinase" of the pathway, Raf. Raf then activates MEK, which in turn phosphorylates MAPK [4]. There exist many different MAPKs and various corresponding upstream

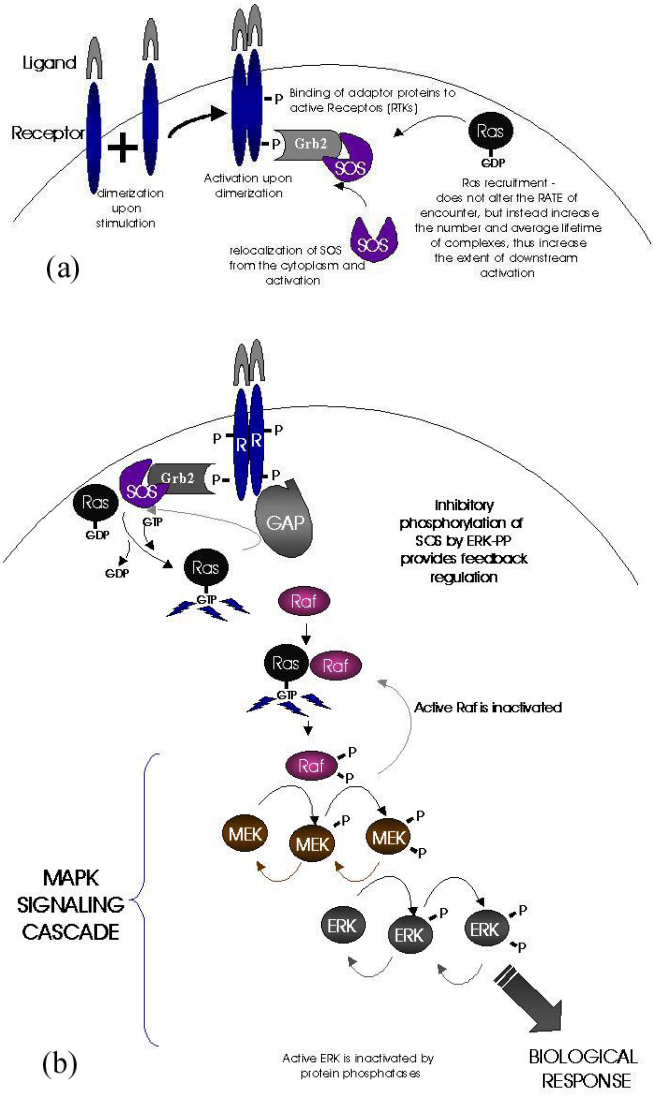

Fig. 1. Schematic representation of Receptor Tyrosine Kinase-activated MAPK signaling cascade. (a) Upon ligand binding RTKs dimerize and recruit adaptor protein Grb2, SOS, and Ras. (b) Activated Ras initiates the firing of three-component MAPK signaling cascade (Raf, MEK and ERK).

kinases in a cell. The generic ERK (extracellular signal-regulated kinase) MAPK has been studied in this simulation.

The activation of the ERK MAPK pathway is alone not sufficient for the cellular response. The duration of the MAPK pathway also contributes to the biological output

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Abstract

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generated. A classic example is the cellular response of a special cell type, called PC12 (for pheochromocytoma cells), which show different response to two different growth factors NGF (nerve growth factor) and EGF (epidermal growth factor), although both growth factors activate the same ERK MAPK pathway. In the case of NGF the cells stop dividing and become neuronal, in the case of EGF the cells divide continuously and stay nonneuronal. It has been experimentally shown that while NGF can maintain sustained activation of the ERK pathway (lasting several hours), EGF can only transiently activate ERK (activation declines to basal levels in about 10 minutes) [5]. It has been previously suggested [6] that the key step in generating the differential kinetics is the regulation of the feedback inhibition of SOS by ERK.

Although this feedback inhibition does indeed have an unquestionable effect on the MAPK signaling in computer simulations, there is no direct biological evidence to indicate that NGF and EGF receptors can indeed regulate this step directly, hence cannot explain how the intrinsic difference of the two pathways arises. We have therefore set out to investigate a more biologically relevant stage at the receptor level.

The receptors may influence downstream effects in a number of ways: one major difference between the two receptors is their level of expression in the cell, i.e. the number of receptor molecules displayed on the cell surface. The number of EGF receptors has been estimated to be around 20,000 receptors per cell, while there are at least 130,000 TrkA molecules (NGF receptors) per cell. It has therefore attracted our attention that the amount of receptor present for each signal can be reflected at the downstream components by recruiting and activating more Ras proteins, thereby more ERK. Our hypothesis has been supported by experimental evidence that when EGF receptors are overexpressed in PC12 cells (i.e. increased in number) they can become neuronal even in response to EGF. We have therefore constructed a signaling pathway using the Grb2 adaptor protein for the EGFR receptor in order to reconstitute the response of these cells when EGFR is overexpressed.

II. Materials and Methods

The standard biochemical kinetics simulation software package GEPASI 3.0 [7] has been used in this study to develop a computer simulation of the EGF signal transduction pathway. On the EGF signal transduction pathway the enzyme-catalyzed reactions follow Michaelis-Menten kinetics, while to simulate all other reactions mass-action kinetics are used. The kinetic parameters used are listed in our web page and are based on experimentally determined parameters (http://www.chemphys.boun.edu.tr /biochem/index.html).

III. Results and Discussion

The intracellular events that take place after receptor stimulation (Fig. 1) have been broken down to discrete steps (Fig. 2). Under conditions where ligand (EGF) concentration has been kept constant (100 nM) the response of the pathway has been studied for different levels of EGF Receptor expression (i.e. different number of receptors per cell) over a period of 60 minutes.

We have initially used the parameters for EGF and EGFR to simulate the transient ERK MAPK activation. As expected, the components of the MAPK cascade, namely Raf, MEK and ERK, show transient activation in the first 2 - 10 minutes, after which they return to basal level of activation (Fig. 3).

Unlike previous reports that receptor number does not affect the duration of the signal [6], we have successfully demonstrated that increase in the number of EGF receptors is sufficient to maintain sustained activation. Fig. 4 (a-c) shows the activation of Raf, MEK and ERK at 60 minutes in response to increasing receptor concentrations, and the

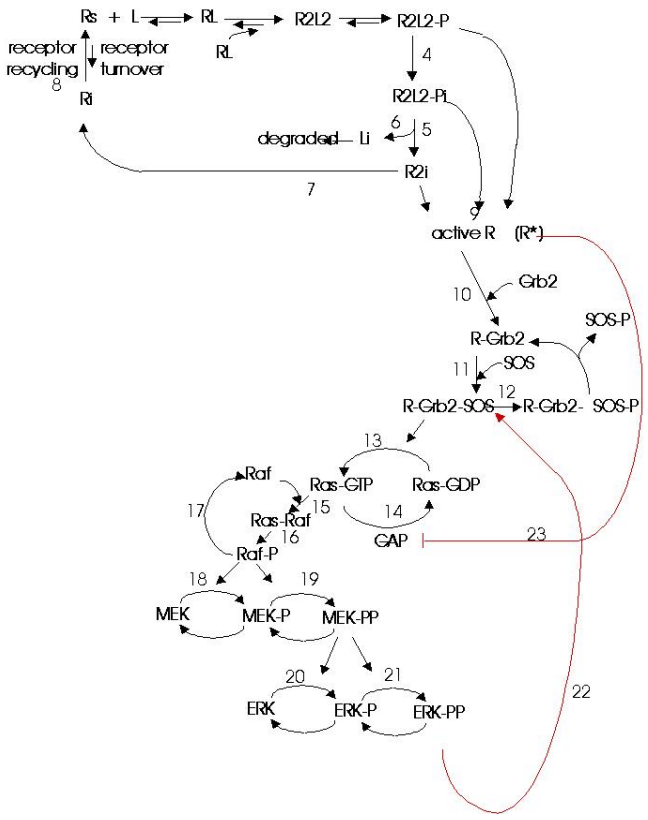

Fig. 2. Stepwise representation of the individual reactions used in the simulation. Numbering is arbitrary, and is included for the sake of simplicity.

profile of this activation over 60 minutes can be found in Fig. 4d. This 10-fold increase is consistent with the number of naturally occurring EGF versus NGF receptors (20,000 versus 130,000) displayed on the cell surface, hence demonstrates the biological significance of our simulation. Whereas Brightman and Fell have regarded the receptor as an enzyme (or modifier) that can be used over and over in the pathway, in biological systems the receptor in fact forms stoichiometric complexes with the adaptor proteins (e.g. Grb2), catalyzes its phosphorylation, and remains associated with that adaptor protein. Our simulation, on the other hand, accounts for the stoichiometry of receptor-mediated Grb2 activation, and considers only the receptor-associated adaptor protein the active form.

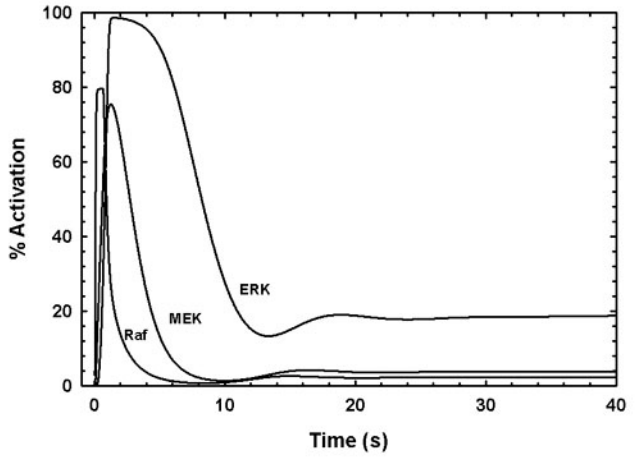

Fig. 3. % Activation of Raf, MEK, and ERK in EGF stimulation, with 11,000 receptors.

IV. Conclusion

The duration of the MAPK signal is of fundamental importance to the final biological response, as is clearly demonstrated experimentally in PC12 cells. Many laboratories have devoted their efforts in trying to establish the mechanism by which NGF and EGF can generate this differential response. Our simulation, consistent with experimental data, suggest that receptor number plays a role in this process, although not likely to be the only factor.

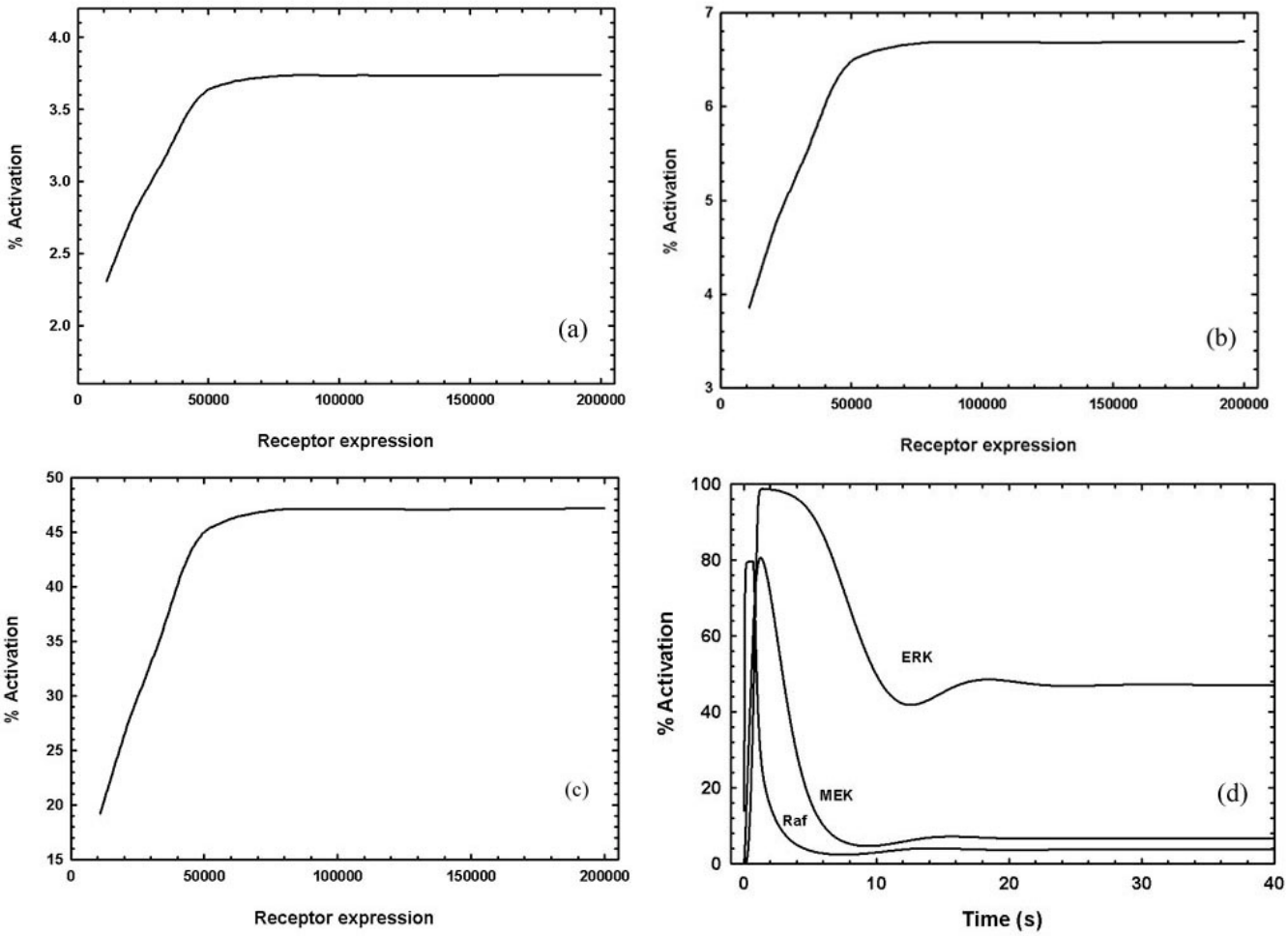

Fig. 4. The effect of receptor overexpression on the steady-state activation of a) Raf, b) MEK, and c) ERK. The activation levels of Raf, MEK and ERK over time is given in (d).

NGF and EGF receptors possess many other properties that are intrinsically different from each other, which will undoubtedly contribute to the kinetics of MAPK activation. Ongoing work is addressed to this issue.

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